



Spectrophotometric Determination of Tetracyclines using Folin-Ciocalteu Reagent

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outline



- Introduction
- Background
- Material and method
- Result and discussion
- Future plan
- Outlook



Introduction



- Quantitative analysis of active ingredients primarily important to indicate quality of products → efficacy of treatment
- Well established and simple analytical methods required by analyst
- Validation of analytical method according to ICH guideline Q2(R1) needed for all new analytical method



Background



- HPLC; standard method for tetracyclines assay
- Spectrophotometric method; simple and less time consuming



Background



- Chromogen; specific and selective
- FCR (Folin-Ciocalteu Reagent)
 - Phenolic compound determination (Everette *et al.*, 2010; Wang and Chou, 2008)
 - Drugs *e.g.* Mesalamine (Chandra *et al.*, 2011)
- Other drug-chromogen complex *e.g.* levodopa-ninhydrin (Patil *et al.*, 2012)

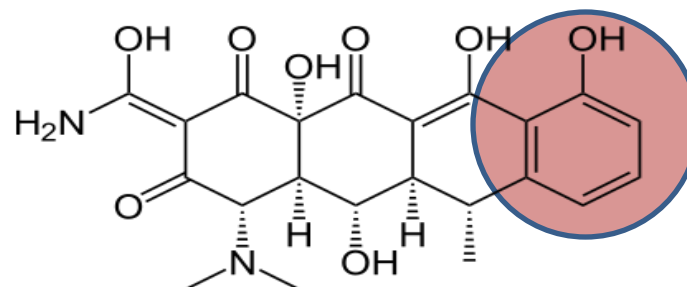
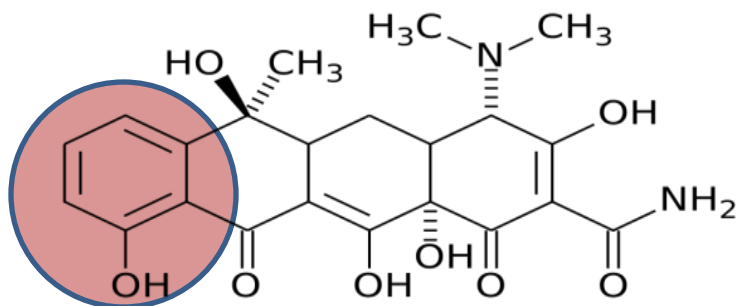


Background

- Tetracycline

Doxycycline

Phenolic group



FCR





Background



- Quality specification according to USP
 - Description, identification, limit test
 - Dissolution, assay

etc.
- Analytical method development
 - Systematic study on factors influencing to analytical method *e.g.* pH
 - Method optimization



Background



- Method validation, ICH guideline Q2(R1)
 - Accuracy
 - Precision
 - Intraday precision (within-day precision)
 - Interday precision (between-day precision)
 - Linearity and range
 - LOD (limit of detection) and LOQ (limit of quantitation)



Material and Method



- Tetracycline, Doxycycline, 0.5 N NaOH, FCR
- Analytical Method Development
 - Effect of 0.5 N NaOH solution on spectrophotometric determination
 - Effect of FCR on spectrophotometric determination
 - Stability of complex



Material and Method



- Method development

Tet, Dox (100 $\mu\text{g/ml}$) 1.0 ml

FCR; 125, 250, 375, 500, 625 μl

0.5 N NaOH; 1, 2, 3, 4, 5 ml

ca. 20 min.

qs c water to 10.0 ml
Abs at 772 nm





Material and Method



- Method Validation

- Accuracy and Precision

$$\%RSD = \frac{SD}{\bar{x}} \times 100$$

- Intraday precision, 6 replicates at low and high concentration (%RSD)
 - Interday precision, 6 replicates at low and high concentration for 3 consecutive days (%RSD)

- Linearity and range; 3 replicates for 5 concentrations (2, 4, 6, 8, 10 µg/ml)

- LOD and LOQ

$$LOD = \frac{3.3\sigma}{S} \quad LOD = \frac{10\sigma}{S}$$



Material and Method



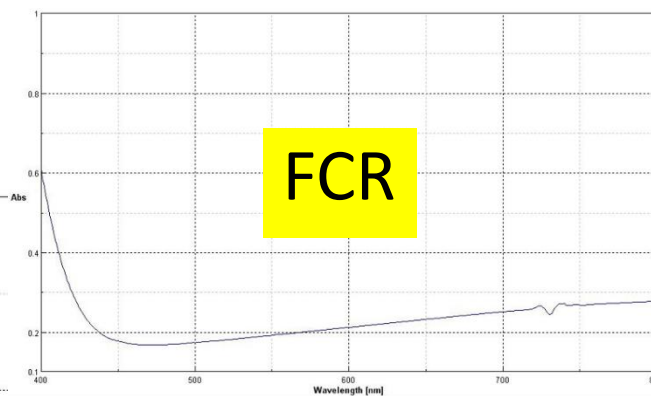
- Method implement
 - Analysis of finished products



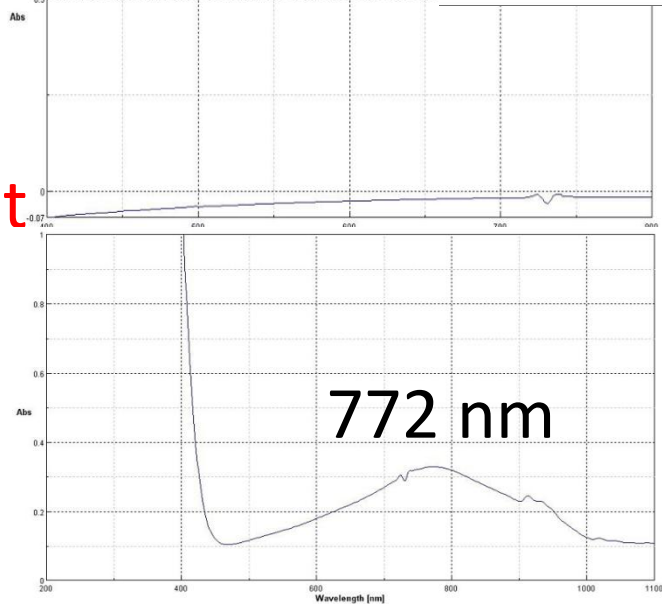
Result and discussion



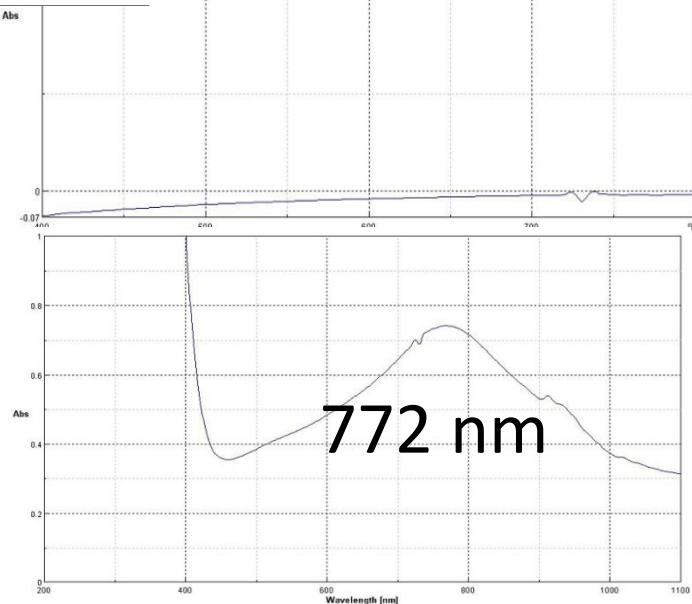
- Screening



Tet



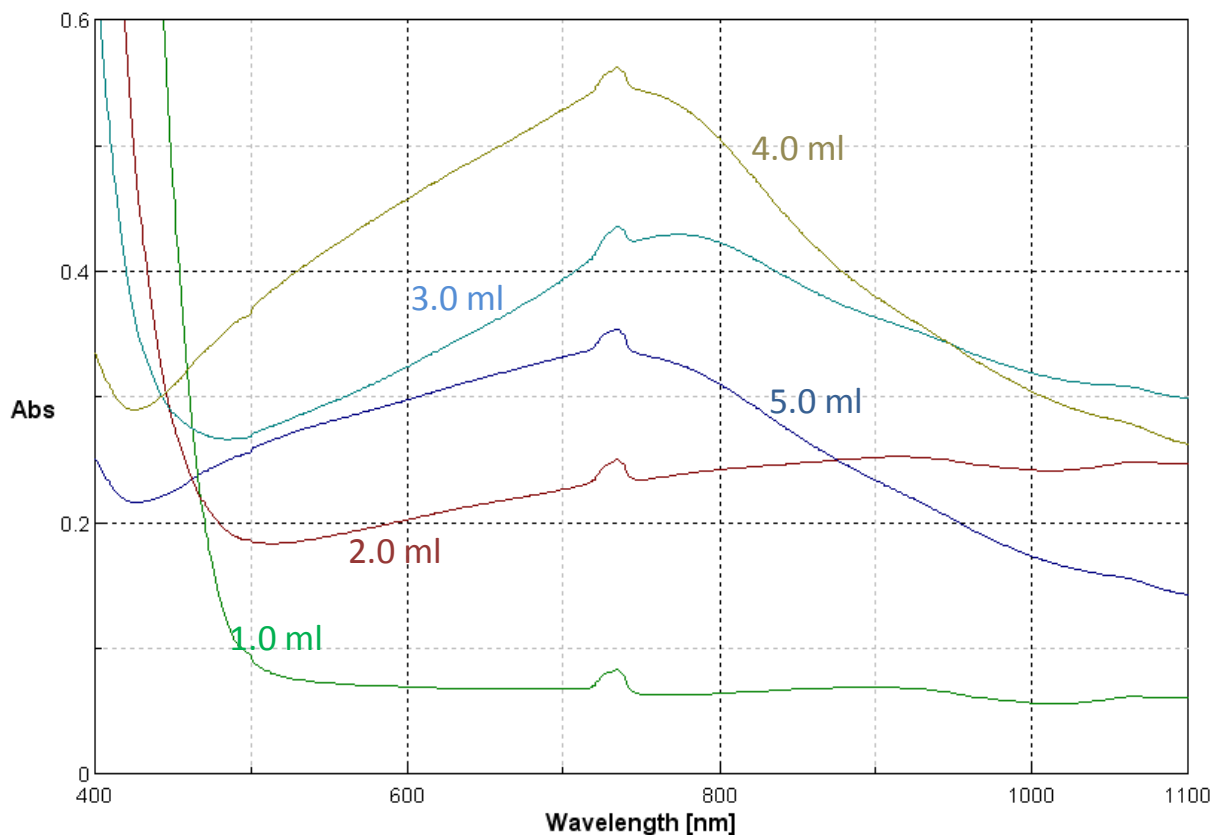
Dox





Result and discussion

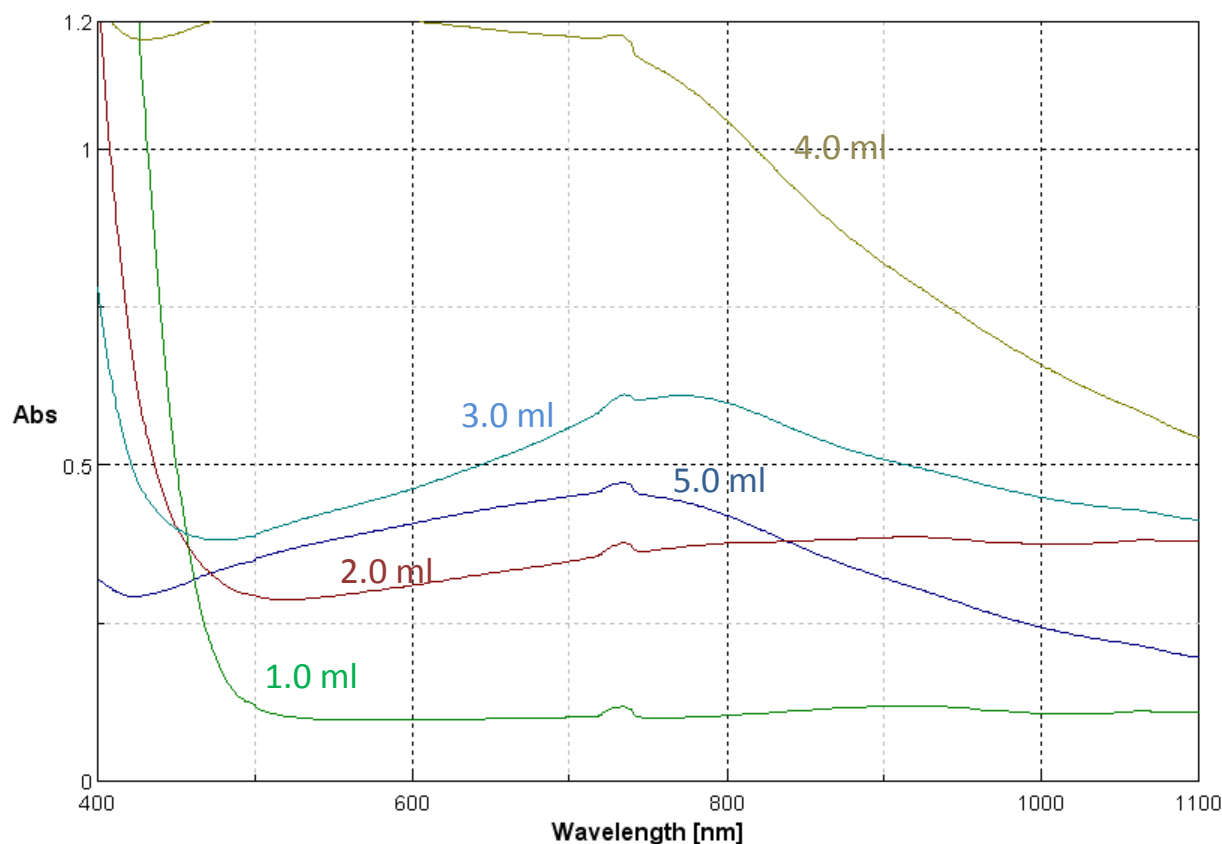
- Effect of NaOH solution on absorbance (1.0, 2.0, 3.0, 4.0, 5.0 ml), **Tetracycline**





Result and discussion

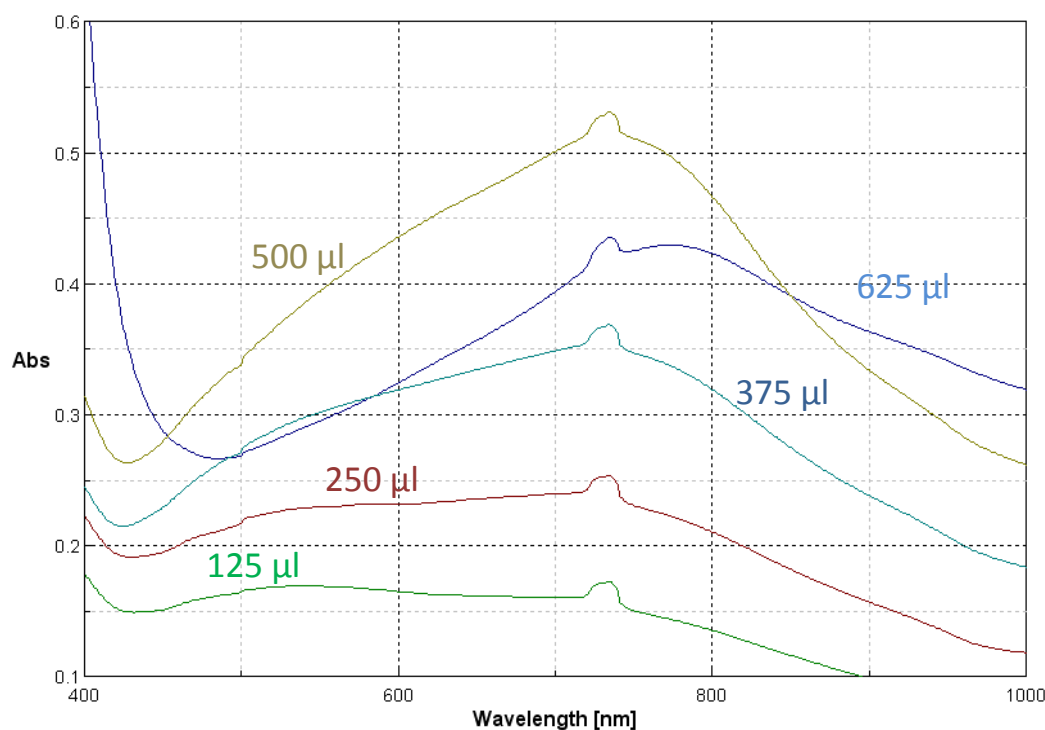
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Result and discussion

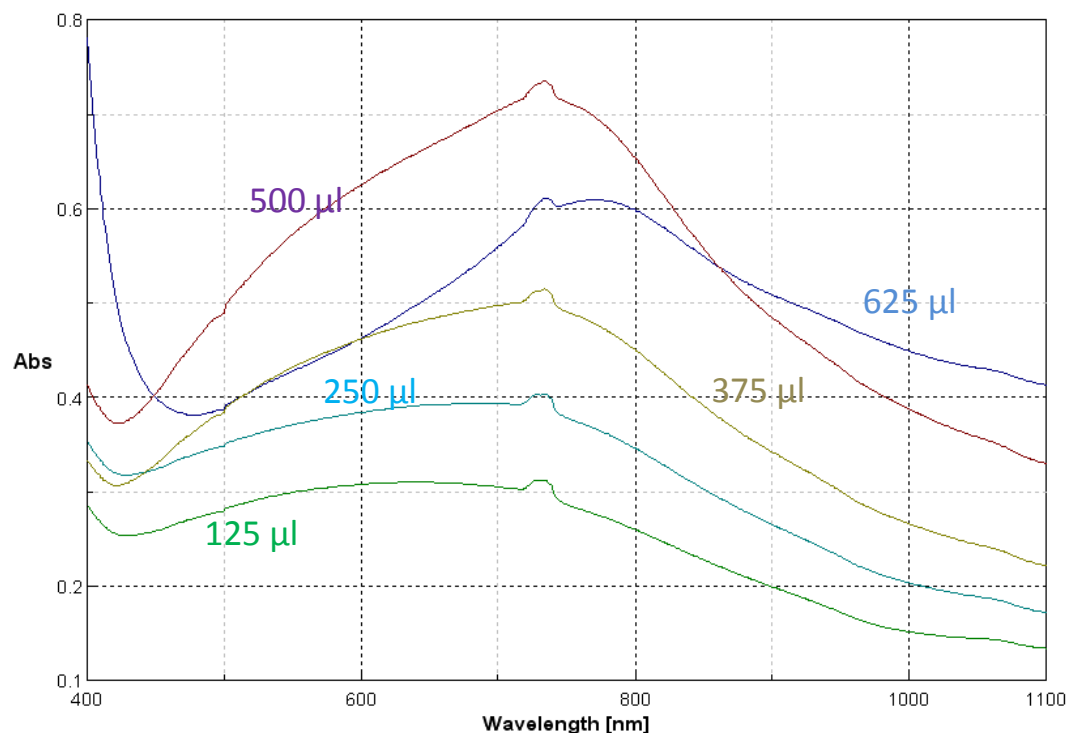
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Result and discussion

- Effect of FCR on absorbance (125, 250, 375, 500, 625 μ l), **Doxycycline**

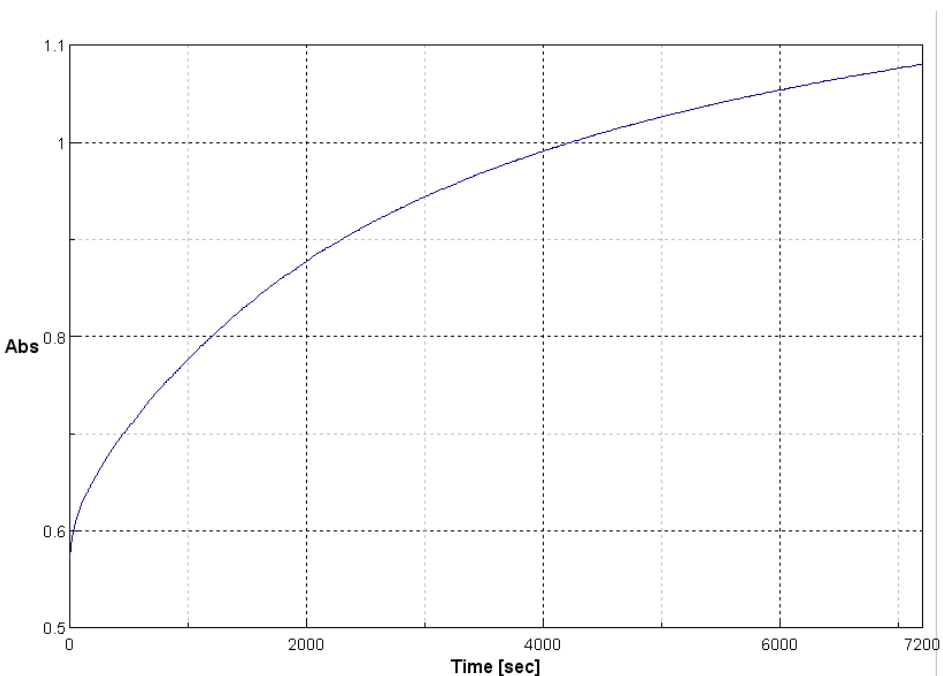




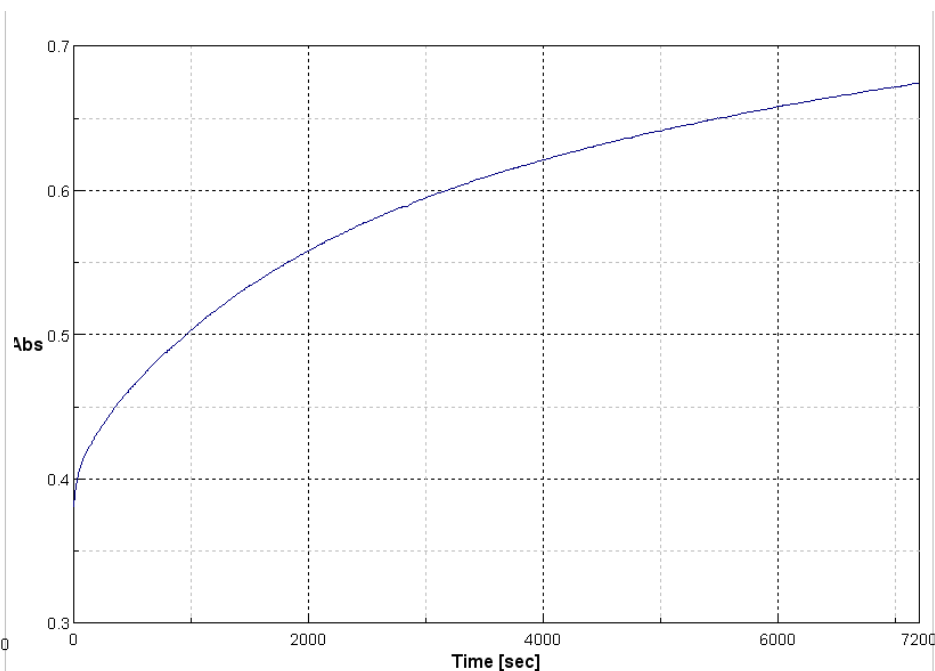
Result and discussion



- Stability of complex



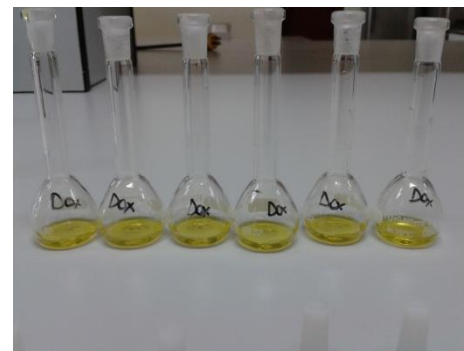
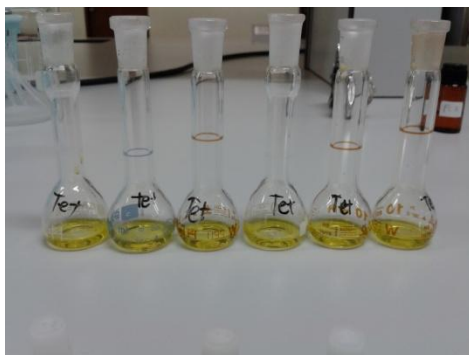
Tetracycline



Doxycycline



Result and discussion





Result and discussion



- Method validation
 - Precision

	Tetracycline		Doxycycline	
	3 µg/ml	7 µg/ml	3 µg/ml	7 µg/ml
Precision				
- Intraday	2.38	3.71	1.87	2.40
- Interday	3.43	5.72	2.86	4.88



Future plan



- Linearity and range (2, 4, 6, 8, 10 $\mu\text{g/ml}$)
 - Equation and coefficient of determination (R^2)
- LOD and LOQ
- Analysis of commercially available tetracyclines



Note



- photometric intensity
 - pH dependent
 - Preparing at once and using the same NaOH solution throughout the measurement
 - Time dependent
 - Promptly measuring after finishing reaction for all sample/standard



Outlook



- Determination of other drugs containing phenolic group using FCR
- pH range and/or temperature on intensity of color formation